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### The cost of living

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**Acclimation and adaptation to different environments: Variations in the specific leaf area and consequences for the metabolic rate**

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## Abstract

The metabolic rate of plants exposed to different temperatures will be affected by the direct, thermodynamic effect of temperature on the rates of enzymatic reactions. By acclimation and adaptation to different temperatures, changes in metabolic (feedback-control of enzymatic processes) and developmental processes will (partly) compensate this direct thermodynamic effect. For plants, the specific leaf area (SLA), reflecting the surface to mass ratio of the leaves, varies between climates, possibly compensating for the effect of temperature on the metabolic rate.

In this study we examine the climate-related differences in SLA and the possible impact of SLA on the metabolic rate comparing individuals of a species (intraspecific) and between species (interspecific). The metabolic rate is positively related to metabolic activity at both the intra- and interspecific level. At the interspecific level we observed climate-dependent differences in SLA. Examination of the individual effects of the different climatic variables, show that temperature has a negative effect on SLA, suggesting that adjustment of the SLA is possibly a compensatory mechanism for the thermodynamic effect of temperature on the basal metabolic rate. However, at the intraspecific level we only observed latitudinal and altitudinal-dependencies for some growth-forms, but not for all plant species. Further, in perennial plant species phenotypic, latitudinal-related differences in leaf size occur. In this case the effect of leaf size on SLA, and thus on metabolic rate, could also be considered a functional, compensatory adjustment to latitudinal effects.

## Introduction

The variation in leaf structure and function reflects differences in plant traits (Reich *et al.* 1997; Reich *et al.* 1999; Hansen *et al.* 2002; Wright *et al.* 2004; Wright *et al.* 2005a; Wright *et al.* 2005b). Depending on ecological requirements for productivity, support and transport, the surface to mass ratio of leaves differs (Niinemets 1999; Westoby *et al.* 2002). The specific leaf area (SLA), the dry mass per unit leaf area, provide a measure of the variation in leaf structure and function (Wright *et al.* 2004). The SLA reflects more than simply the leaf area to surface ratio; it is related to plant traits and internal functioning. For instance, SLA is related to nitrogen content (Reich *et al.* 1997; Poorter & Evans 1998; Reich *et al.* 1999; Wright *et al.* 2004), and reflects differences in the metabolic activity and relative growth rate of plants. Thin leaves possessing a low SLA, are an indication for high metabolic activity, both in photosynthesis and in respiration (Reich *et al.* 1997; Poorter & Evans 1998; Reich *et al.* 1999; Wright *et al.* 2004; Lee *et al.* 2005). Thus differences in SLA could provide a regulatory mechanism for variations in metabolic activity in different environments. Differences in SLA might provide e.g. a compensatory mechanism for the thermodynamic effect of temperature on the metabolic activity. This thermodynamic effect is an increase in the rate of enzymatic reactions, as with higher temperatures less of the substrate molecules possess enough energy to attain the transition state of the reaction. Upon extended exposure to a different temperature, adjustment in physiology and/or morphology can compensate for the direct effect of temperature on the metabolic activity (Tjoelker *et al.* 1999; Atkin *et al.* 2000; Xiong *et al.* 2000; Hochachka & Somero 2002; Atkin & Tjoelker 2003; Gifford 2003; Atkin *et al.* 2008). Differences in morphology, in addition to the feedback regulation of enzymatic processes, could regulate the metabolic activity of plants in different climates (Hochachka & Somero 2002; Loveys *et al.* 2003; Atkin *et al.* 2006a). To compensate for the thermodynamic effect of temperature, plants from low temperature are expected to have a high SLA to counterbalance the temperature-induced decreased metabolic activity.

Studying the SLA in different climates, one should consider that the SLA is sensitive to several different aspect of climate, such as temperature, solar radiation, as well as water and nutrient availability (Tjoelker *et al.* 1998b; Wright *et al.* 2004; Wright *et al.* 2005a; Atkin *et al.* 2006a; Poorter *et al.* 2009). High solar radiation or mechanic influences such as rainfall or herbivory result in a low SLA. In contrast high water and nutrient availability is accompanied by a high SLA (Woodward 1983; Bolstad *et al.* 1999; Turnbull *et al.* 2003; Wright *et al.* 2006; Poorter *et al.* 2009). The effect of temperature on the SLA is under dispute, since different studies showed different effects (Wright *et al.* 2006; Poorter *et al.* 2009). Comparing climatic differences on a global scale, at low latitudes solar radiation is high, while rainfall and nutrient availability are low (Barry & Chorley 1992). The impact of the various environmental parameters could change the SLA in a similar direction, e.g. high temperature and solar radiation, as well as low rainfall and nutrient availability result in a low SLA at

low latitudes. However, the effect of temperature is unclear, so that the opposite effect, an increased SLA at low latitude, would counterbalance the effects of solar radiation, rainfall and nutrient availability. In case environmental parameters have similar effects on the SLA the climatic differences in SLA are stronger, while counter-acting effects on the SLA would result in a weak dependence of SLA on climate.

Differences in the SLA of plants from different climates could be the result of either acclimation or adaptation processes. At the interspecific level, comparing plant species from different climate zones, one can detect adaptive differences, while at the intraspecific level acclimation to local environmental conditions of individuals of a single plant species occurs (Chapter 2). Differences in SLA have mainly been studied between species adapted to different climates. The effect of SLA on differences in metabolic activity as well as climatic differences in SLA on plant species acclimated to different conditions is hardly studied.

The SLA also differs between different taxonomic groups, e.g. deciduous and evergreen angiosperms and gymnosperms, or different growth-forms, e.g. annual and perennial herbaceous as well as woody plant species (Wright *et al.* 2005a; Price & Enquist 2007; Poorter *et al.* 2009). Evergreen angiosperms and gymnosperms are adapted to low water and/or nutrient availability which is accompanied by high leaf life-span and a low SLA. Further, the SLA can vary for plants differing in the length and time of their growing season. A shift in growing season to earlier or later in the year would result in different environmental conditions during growth and thus in differences in the SLA (Reich *et al.* 1997; Poorter & Evans 1998; Wright *et al.* 2005a). Furthermore differences in the SLA depend on leaf size. The biomass investment per unit leaf area can differ, depending on the ratio between productive and supportive tissue, which in turn is related to leaf size. (Niklas *et al.* 2007; Milla *et al.* 2008). Therefore, differences in leaf size across biomes might be accompanied by differences in SLA.

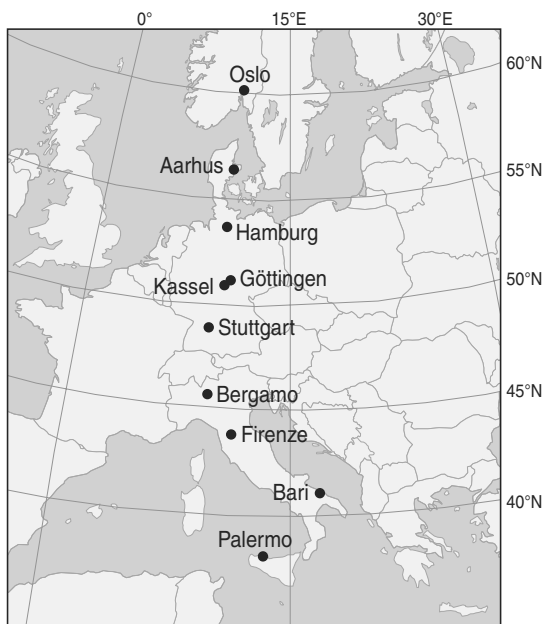
In this study the metabolic rate and SLA of plant species acclimated to different growth temperatures and between species adapted to different climates were compared. To determine the impact of SLA in different climates on the metabolic rate, we studied the correlation between climate and SLA, and between SLA and metabolic activity. Firstly, we show the effect of SLA on the metabolic activity and differences in the SLA between taxonomic groups and growth-forms. Secondly we highlight the effect of latitude on SLA and leaf size for single species and between species. Subsequently, we attempt to separate the effects of environmental parameters such as temperature, solar radiation, and precipitation. This approach might provide a mechanistic explanation for differences in the SLA between different growth-forms and in different climates.

## Material and methods

### Design of the study

Nine evenly distributed locations along a south-north gradient in Europe were selected for sampling (Figure 5.1, Table 5.1). The sampling took place from July till September in 2007. Samples were collected in the botanical gardens to reduce the effects of local differences in water and nutrient availability, as in these gardens plants are provided with sufficient water and nutrient rich soil. The sampling sites differ in their mean growing season temperature and prevailing temperature during the sampling period (Table 5.1). Mean growing season temperature and prevailing temperature are positively related to each other ( $N = 9$ ,  $R^2 = 0.65$ ,  $p > 0.01$ ); in our analysis we used the prevailing temperature, since plants are likely to have adjusted to this temperature (Table 5.1). In addition to the botanical garden in Kassel, the botanical garden in Goettingen, which is nearby was sampled to compensate for the limited number of available samples in Kassel.

Our sampling also includes non-native species, since several species, originating from different regions around the world, have been established in Europe in the last century and therefore can be regarded as adapted. To ensure that plants have acclimated/adapted to the prevailing conditions, we only sampled plants growing outside (no greenhouse cultures). Ecotypes of species were registered, but not treated as



**Figure 5.1** Map of the sample sites of the south-north transect across Europe. Further details to the sample sites are shown in Table 5.1.

different species. In contrast, hybrids of species are treated as different species. In total we took 3538 samples from 365 species including 309 deciduous, 43 evergreen and 13 gymnosperm plant species. For the determination of the species-specific temperature-dependencies of the SLA, a species was required to be available at, at least, three different locations. The four species used for the intraspecific temperature-dependency of the respiration were available at, at least, seven different locations. For the between species temperature-dependence of the SLA and respiration, all samples were taken into account, independent of the number of locations the species was available at. For the measurements three to five full-grown leaves of each species were sampled. To make sure that the leaves were full-grown the plastochron of the sampled leaves had to be above three. In addition the color and shape of the leaf was taken into account to evaluate the growth stage.

### Respiration measurements

To determine the intraspecific dependence of the respiration on temperature and SLA, respiration measurements were done on four species, including *Aesculus hippocastanea*, *Corylus avellana*, *Hedera helix* and *Quercus ilex*. For the between species dependence of the respiration on the SLA of six species from single locations were included: *Citrus decamona*, *Fagus sylvatica*, *Quercus palustris*, *Quercus rubra*, *Quercus robur* and *Quercus turnerii*. Oxygen consumption was measured on at least three samples in the dark at the prevailing temperature (Table 5.1) on leaf discs of approximately 10–15 cm<sup>2</sup> in a cuvette with a volume of 50 ml. The oxygen was measured optically (Fibox 3, PreSens). In addition, the temperature sensor of the Fibox 3 attached to the cuvette, measured the prevailing temperature.

Location	lat (°N)	lon (°E)	Temperature in °C	Solar radiation per hour	Precipitation (GS) in mm	Season (GS)
Palermo	38.1	13.1	29.3	0.38	611	all year
Bari	41.1	16.5	31.5	0.33	657	all year
Firenze	43.5	11.1	27.8	0.30	911	all year
Bergamo	45.4	9.4	21.1	0.34	1029	feb-nov
Stuttgart	48.5	9.1	20.7	0.28	551	mar-nov
Kassel	51.2	9.3	19.7	0.25	526	mar-nov
Goettingen	51.3	9.6	19.7	0.25	486	mar-nov
Hamburg	53.4	9.6	23.0	0.24	589	mar-nov
Aarhus	56.2	10.2	23.4	0.25	489	apr-nov
Oslo	59.5	10.4	22.2	0.27	507	apr-oct

**Table 5.1** Geographical coordinates and climatic variations of the nine sampled sites along the sampled south-north transect.



For the analysis of respiration we used the prevailing temperature at the location. The respiration is calculated per unit dry weight (g) from the measured leaf discs. The measured respiration rates were transformed from gas units ( $\mu\text{mol/g/s}$ ) to energy units (Watt/g), by multiplying by a coefficient of 0.47 (Boeger 1978), assuming that plants are adjusted to the prevailing environmental conditions, and not stressed so that energy is obtained from phosphorylating pathways (Hansen *et al.* 2002; Moore *et al.* 2002). In case non-phosphorylating pathways are also active, it is assumed, that the distribution of energy obtained from phosphorylating and non-phosphorylation pathways (lower energy yield) does not differ between the locations, so that the calculated values for energy consumption are systematically overestimated and would not affect our results.

### **Leaf size/Specific leaf area**

To determine the leaf area and biomass for each leaf, the leaves got an identification mark. Furthermore leaves were photographed, together with a calibration scale, within five hours after sampling with a digital camera (HP FCLSD-0402), to determine the leaf area using the image analysis software SigmaScan Pro5. For storage during sampling the leaves of each species at each location were preserved separately between blotting paper. After the field-sampling the leaves were dried in a stove for at least 48 h at 80°C and subsequently the leaf dry weight was determined, using a balance (Sartorius 1712). For the analysis of the temperature-dependence of the SLA we used the mean growing season temperature as leaves had developed during the growing season as most plants are deciduous and shut down their metabolism during winter time (Li *et al.* 1998). The growth season temperature is calculated from available climate data ([www.klimadiagramme.de](http://www.klimadiagramme.de)) as the average of the months in which the temperature is above 4°C.

### **Calculations and statistical analysis**

Because respiration is exponentially related to temperature and SLA, and leaf size, is presumed to be exponentially related to environmental parameters, we ln-transformed these data to obtain a linear relation. Furthermore, we ln-transformed data on geographical coordinates to the natural logarithm, because we observed a better linear fit of solar radiation and temperature to ln-transformed geographical coordinates.

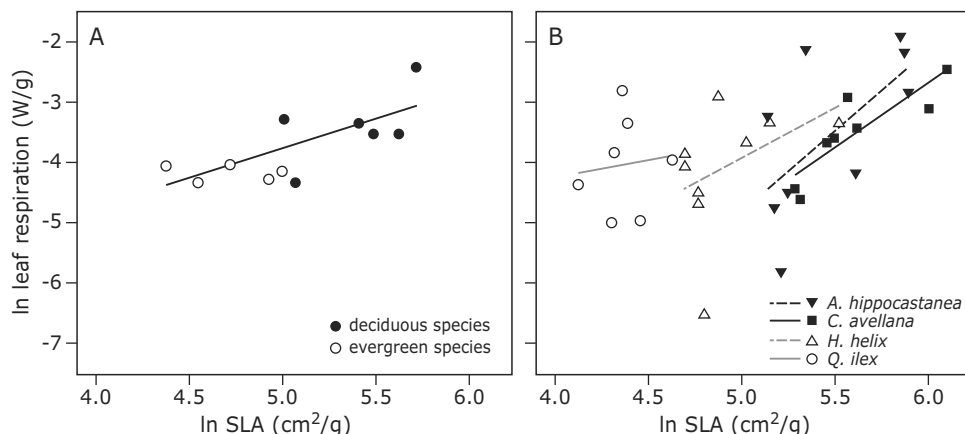
For the analysis of interspecific data we calculated the average of respiration, SLA, leaf size, latitude, altitude and environmental variables of all locations, where the species was sampled, so that each species is always represented as a single data point. For the intraspecific analysis we calculated the average of SLA and leaf size at each location, because of variations in the sample size of a species at different locations.

Intra- and Interspecific data of ln respiration and ln SLA are normal distributed, but not data on ln leaf size, which are only visually normal distributed (the distribution was skewed to the right). First we analysed the interspecific dependence of ln

respiration on  $\ln$  SLA applying a simple regression analysis. To determine the intraspecific dependence, we applied a GLM, homogeneity of slopes, to analyse differences between the dependencies and subsequently an univariate ANCOVA, as the dependencies of  $\ln$  respiration on  $\ln$  SLA do not differ. To detect differences in the SLA between plant categories (deciduous and evergreen angiosperms and gymnosperms) and locations we applied an ANOVA for each category and a nested ANCOVA on the average of the SLA at each location of all species; the location is nested in the plant category. Furthermore we applied an ANOVA for deciduous angiosperms to determine differences in the SLA between growth-forms: annual herbaceous, perennial herbaceous and perennial woody plants. For the interspecific analysis we tested for differences in the dependencies between growth-forms using a GLM on the homogeneity of slopes. Further, we applied an ANCOVA to determine the dependence of  $\ln$  SLA on  $\ln$  latitude and  $\ln$  altitude or on the different environmental variables (growing season temperature, growing season solar radiation and growing season rainfall). For the intraspecific analysis we also applied a GLM, homogeneity of slopes, to determine differences between the dependencies of the various included species. Subsequently we applied an ANCOVA if the dependencies did not differ and a multivariate ANCOVA, separate slope model, when they did. We applied the analysis to all species and further separated the data into annual herbaceous species, perennial herbaceous species and perennial woody species to determine the effects for different growth-forms. We also applied an ANCOVA to determine the effect of the different environmental parameters in parallel. For comparing the intra- and interspecific results, we considered deviations from the 95% confidence interval of the observed slopes.

## Results

The results show that the leaf respiration at the intra- and interspecific level is independent of the prevailing temperature, (Regression analysis (inter):  $N = 11$ ,  $R^2 = 0.16$ ,  $p = 0.25$ , multivariate ANCOVA (intra):  $Df = 4$ ,  $F = 1.00$ ,  $p = 0.43$ ), indicating that the plant species, by adaptation and/or acclimation, adjusted to the prevailing temperature. The analysis of the dependence of respiration on SLA, at the prevailing temperature, shows that for different plant species leaf respiration is related to SLA (Regression analysis:  $N = 11$ ,  $R^2 = 0.56$ ,  $p < 0.05$ , Figure 5.2A). The analysis of the dependence of respiration at the intraspecific level for the four species also shows that respiration is dependent on SLA (multivariate ANCOVA:  $Df = 4$ ,  $F = 3.12$ ,  $p < 0.05$ , Figure 5.2B) and that the dependencies do not differ between species (multivariate ANCOVA:  $Df = 3$ ,  $F = 0.31$ ,  $p = 0.82$ ). The observed positive regression fits reflect that respiration increases with increasing SLA for both the intra- and interspecific level and that the relations do not differ between different plant species. Comparing the intraspecific to the interspecific dependencies of leaf respiration on SLA shows that the slopes are similar (Kruskal-Wallis test:  $H(1, N = 5) = 0.5$ ,  $p = 0.48$ ), so that the effect



**Figure 5.2** Intra- and interspecific dependence of dry mass standardized leaf respiration on specific leaf area (SLA);

(A) interspecific, each point represents a species adapted to a distinct environment, deciduous species and evergreen species.

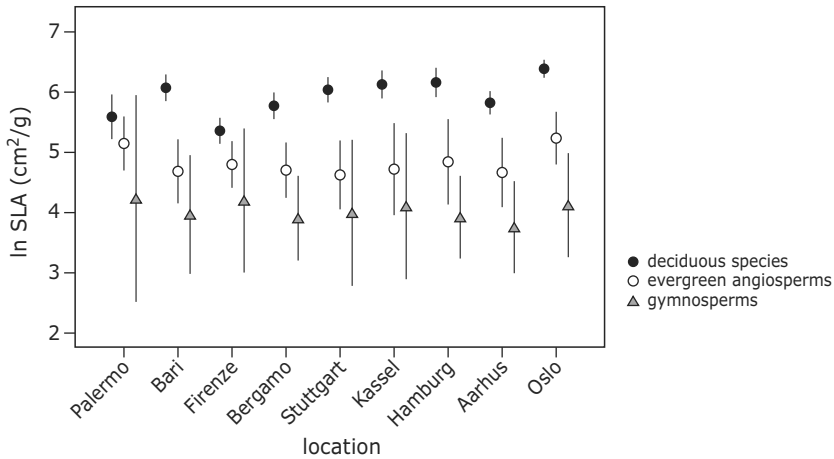
(B) intraspecific dependencies, the symbols represent different species deciduous species: *A. hippocastanea* and *C. avellana*, evergreen species: *H. helix* and *Q. ilex*.

of SLA on metabolic activity does not differ comparing individuals of a single plant species or comparing different plant species.

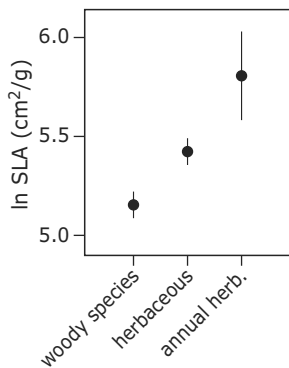
The analysis of the SLA of deciduous and evergreen angiosperms and gymnosperm plants shows that the SLA is lowest for gymnosperms and highest for deciduous angiosperms (Figure 5.3, nested ANCOVA: Df = 18, F = 14.59,  $p < 0.0001$ ). In addition the SLA of deciduous plants varies at the different sampled locations, indicating that deciduous plants are more sensitive to environmental differences (ANOVA: Df = 8, F = 9.36,  $p < 0.0001$ ) than evergreen angiosperms (ANOVA: Df = 8, F = 1.12,  $p = 0.36$ ) and gymnosperms (ANOVA: Df = 8, F = 0.22,  $p = 0.98$ ). Because environmental differences hardly affect the SLA of evergreen angiosperm and gymnosperms, we exclude them from the further analysis.

The SLA varies between different growth-forms of deciduous plants (annuals and perennial herbaceous, shrubs and trees, Df = 3, F = 17.12,  $p < 0.0001$ , Figure 5.4). The post-hoc analysis showed that the SLA of shrubs and trees do not differ from each other, so that we combined those data in the category woody plants. Thus in the further analysis three categories were recognized: annual and perennial herbaceous and woody plants.

For plants the relative investment in supportive tissue changes with increasing leaf size, so that differences in leaf size are accompanied by differences in the SLA. At the interspecific level we indeed observed dependencies of SLA on leaf size, which is similar for the three different growth-forms of deciduous plants (Figure 5.5A, Table 5.2). Because of the dependence of SLA on leaf size, further analyses were done,

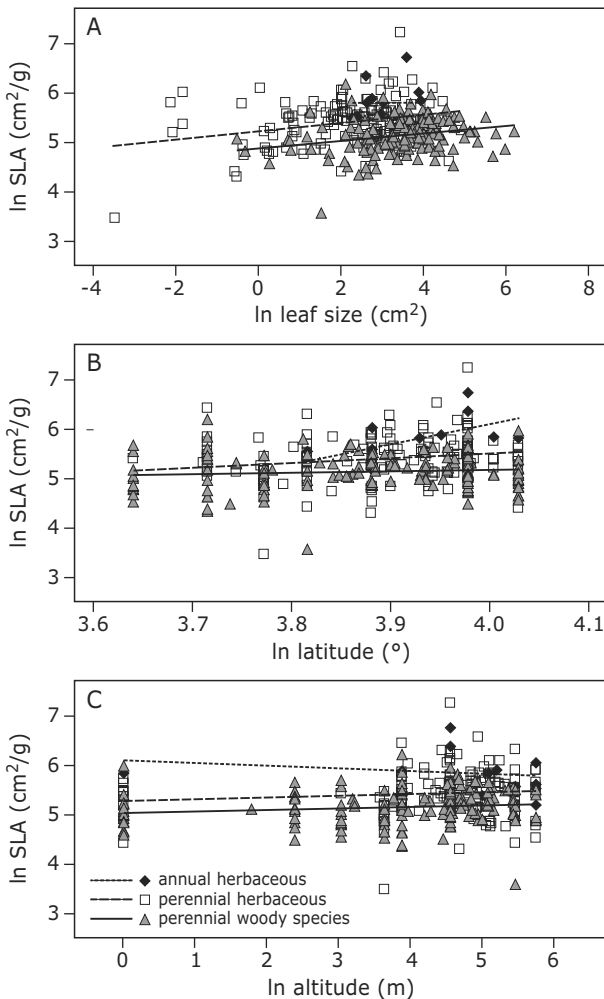


**Figure 5.3** Variations in the ln-transformed specific leaf area (SLA) at the sampled sites from south (Palermo) to north Europe (Oslo) separated to the taxonomic groups: deciduous and evergreen angiosperms and gymnosperms. Symbols show the median SLA of the plants sampled at each location and the error bars mark the 95% confidence interval.



**Figure 5.4** Differences in SLA between the different growth-forms of deciduous plants. Symbols show the median of the observed SLA and the error bars mark the 95% confidence interval.

normalizing the data for leaf size. The interspecific analysis of deciduous plants adapted to different environments shows that the SLA varies between latitudes (Figure 5.5A, Table 5.2) and altitudes (Figure 5.5B). The latitudinal and altitudinal-dependence of the SLA do not differ between growth-forms (Table 5.2), so that for the annuals and perennials herbaceous, as well as woody plants the SLA depends on climate in similar ways. The increase in SLA at high latitudes indicates that adaptation to cold climates is accompanied by thinner leaves, providing high metabolic rate. To



**Figure 5.5** Interspecific dependence of  $\ln$  SLA on (A)  $\ln$  leaf size, (B) latitude and (C)  $\ln$  altitude. The symbols mark the observed values for the different growth-forms: annual herbaceous, perennial herbaceous and perennial woody species. Statistics are given in Table 5.2.

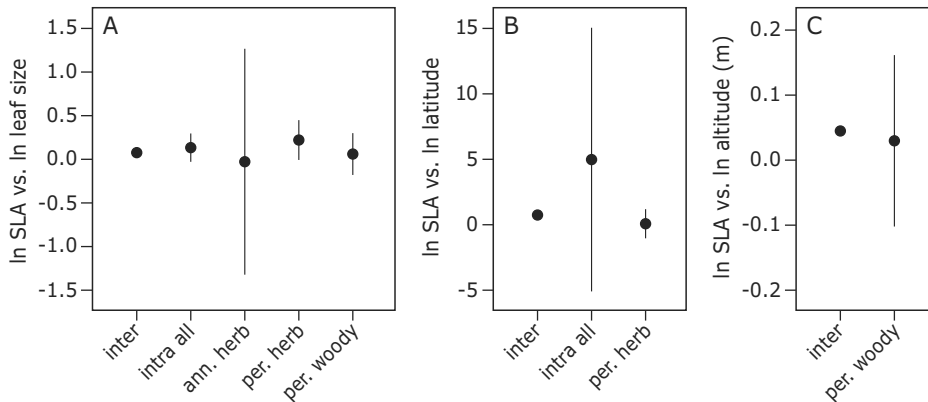
determine the effect of environmental conditions we analysed the effects of growing season temperature, growing season solar radiation and growing season precipitation on leaf size-normalized SLA. Our results show that the climatic effect on SLA is the sum of the positive correlation with solar radiation and the negative correlation with temperature and precipitation (Table 5.2). The strong impact of temperature on SLA is reflected in the high significance of its effect. As SLA is related to latitude, one possibility is that the leaf size depends on latitude, too. However, we did not observe dependencies of leaf size on latitude (Table 5.2), implying that only SLA, but not leaf size is related to climate.

Variable			Analysis							
Dependent	Predictor		ANCOVA					Homogeneity of slope		
	Categorical	Continuous	Df	F	P	B	95%	Df	F	p
ln SLA	growth-form	ln leaf size	1	20.17	<0.0001	0.079	±0.035	2	0.06	0.94
		ln latitude	1	10.03	<0.005	0.731	±0.454	2	1.94	0.15
		ln altitude	1	6.77	<0.01	0.043	±0.032	2	0.62	0.54
		temperature	1	14.57	<0.0005	-0.152	±0.078	2	1.73	0.18
		precipitation	1	10.92	<0.005	-0.001	±0.0004	2	0.04	0.96
		radiation	1	10.24	<0.005	7.383	±4.539	2	0.16	0.85
ln leaf size	ln latitude	1	0.63	0.43						

**Table 5.2** Statistics and the regression coefficients of interspecific data, including 95% confidence-interval, for the dependence of ln SLA on ln leaf size and geographical coordinates and the dependence of ln leaf size on latitude. The ANCOVA indicate significant dependencies ( $p < 0.05$ ) and the statistics on the homogeneity of slope shows differences in the dependencies between growth forms ( $p < 0.05$ ).

At the intraspecific level, our results show again, that the SLA is related to leaf size, while the dependence of SLA on leaf size varies between species (Table 5.3). Further we tested if the differences might depend on the different growth-forms. The leaf size is related to SLA for annual and perennial herbaceous as well as for woody plant species (Figure 5.6A, Table 5.3). The relation also varies only considering annual herbaceous or woody plant species, so that in this case the dependence of SLA on leaf size is species-specific. We did not observe species-specific differences in the dependence of SLA on leaf size for perennial herbaceous, so that for this growth-form the dependencies are similar. Comparing our intra- and interspecific results, the dependence of SLA on leaf size does not differ (Figure 5.6A). Thus the impact of leaf size on SLA is similar for individuals of a single species and between species.

Again, we did the further analysis on leaf size-normalized data. At the intraspecific level, the SLA has the tendency to depend on latitude and is independent of altitude for all species, a result which might be the consequence of combining different growth-forms (Table 5.3). We separated the plant species into the three different categories and tested for the dependence of SLA on latitude and for differences between the dependencies of the plant species within each of the groups. Our results show that for annual and perennial herbaceous plants the SLA depends on latitude, a dependence which is similar between species, while for woody plant species the SLA only has a tendency to depend on latitude (Figure 5.6B, Table 5.3). Further, the SLA depends on altitude for woody, plant species and do not differ between the species of each group (Figure 5.6C, Table 5.3). Conclusively, the dependence of SLA on latitude and



**Figure 5.6** Box-plots on the intra- and interspecific dependencies of (A) ln SLA on ln leaf size, (B) ln SLA on ln latitude, and (C) ln SLA on ln altitude. The symbol marks the median slope, and the error bars show the 95% confidence interval of the observed slopes. We only show intra- and interspecific data as well as growth-forms where we observed a significant dependency. The interspecific dependencies do not differ between growth-forms. The interspecific slopes are within the 95% confidence intervals for the intraspecific slopes of all species and of the growth-forms we observed a significant dependence.

altitude differs between growth-forms and can not be generalized for intraspecific data. Separation of the climatic effects on leaf size-normalized SLA into growing season temperature ( $Df = 24$ ,  $F = 1.01$ ,  $p = 0.48$ ), solar radiation ( $Df = 24$ ,  $F = 0.69$ ,  $p = 0.83$ ) and precipitation ( $Df = 24$ ,  $F = 1.14$ ,  $p = 0.36$ ), did not result in significant correlations of the intraspecific differences in SLA with these environmental variables. This shows that differences in the SLA are the result of the combined change in environmental variables. The impact of single environmental variables on the analysed plant species seems to differ between the variety of plant species, so that some might be susceptible for temperature while others are susceptible for solar radiation or precipitation. Therefore, we observed a latitudinal effect, but could not determine the single effects of environmental variables.

In contrast to interspecific results, at the intraspecific level leaf size is dependent on latitude, an effect which differs between plant species (Table 5.3). Further we tested for leaf size-dependence on latitude for the different growth-forms. For perennial herbaceous and woody plant species the leaf size is related to latitude, but not for annual herbaceous plant species. These results show that phenotypic, climate-dependent differences can occur not only in SLA, but also in leaf size. Further the leaf size-dependencies between plant species of a distinct growth-form are similar. Therefore, the observed differences in the dependence of leaf size on latitude combining all species might result from the differences between growth-forms.

Variable			Analysis					
Dependent	Predictor		ANCOVA			Homogeneity of slope		
	Categorical	Continuous	Df	F	p	Df	F	p
ln SLA	species	ln leaf size	73	1.96	<0.0005	72	1.73	<0.005
	annual species		4	5.29	<0.05	3	5.81	<0.05
	herbaceous species		1	23.26	<0.0001	38	1.29	0.16
	woody species		30	1.69	<0.05	29	1.72	<0.05
	species	ln latitude	73	1.35	0.08			
	annual species		1	8.67	<0.05	3	2.55	0.17
	herbaceous species		1	13.09	<0.0005	38	1.24	0.20
	woody species		1	1.38	0.24			
	species	ln altitude	1	0.50	0.48			
	annual species		1	0.28	0.61			
	herbaceous species		1	1.25	0.27			
	woody species		1	6.18	<0.05	29	0.90	0.62
ln leaf size	species	ln latitude	73	1.76	<0.005	72	1.50	<0.05
	annual species		1	0.23	0.64			
	herbaceous species		1	4.43	<0.05	38	1.30	0.15
	woody species		1	27.56	<0.0001	29	1.60	0.06

**Table 5.3** Regression analysis (GLM) of the intraspecific data on the dependence of ln SLA on geographical coordinates and ln leaf size as well as the dependence of leaf size on latitude. The regression analysis is separated to all plant species and for the different included growth-forms, annual herbaceous, perennial herbaceous species and perennial woody species. The ANCOVA shows significant dependencies ( $p < 0.05$ ) for the different species and the GLM on the homogeneity of slope indicate significant differences ( $p < 0.05$ ) between the dependencies for the included species.

## Discussion

Our results show that by acclimation to different temperatures, the respiration becomes temperature-independent and cannot be predicted by the thermodynamic effect on enzymatic reactions. Thus our study confirms earlier studies that questioned the thermodynamic-dependence of the metabolic rate on temperature (Tjoelker *et al.* 1998b; Xiong *et al.* 2000; Atkin *et al.* 2008). Furthermore our results confirm that respiration is dependent on SLA, when comparing various plant species, interspecific level (Reich *et al.* 1999; Wright *et al.* 2004; Wright *et al.* 2005b; Galmes *et al.* 2007). Here we additionally show that SLA-dependent differences in the metabolic rate also apply for individuals of a single plant species, acclimated to different climates. Thus, differences in SLA between different climates might provide a compensatory mechanism for the thermodynamic impact of temperature on the metabolic rate.



For plant species adapted to higher latitudes, and thus to colder climates, the SLA is increased. Similar results were shown in earlier studies, comparing the SLA of deciduous tropical and arctic plants (Reich *et al.* 1999; Wright *et al.* 2006; Galmes *et al.* 2007). Compared to our study, covering a range of growing seasons from 5.4–18.5°C, the earlier study even considered yearly temperatures ranging from approximately -15°C to +30°C. In contrast, a recent study shows that plants have an increased SLA in high temperature (Poorter *et al.* 2009). In the present study we also considered the effect of leaf size on the SLA and we only included deciduous plants, so that the differences in the results might be the effect of differences in the datasets.

Moreover, differences in the SLA between climates are the result of combined environmental effects on SLA. Here we showed that temperature and the impact of precipitation have opposite effects to solar radiation. The resulting overall effect of the different environmental parameters is an increase in the SLA in colder climates. Since water and nutrient availability is increased in high latitudes, including these variables would not lead to different results. The increase in SLA in cold climates, accompanied by an increase in metabolic activity, shows that climate-induced changes in plant architecture can counteract the thermodynamic effect of temperature on the metabolic activity.

In addition, our results on intraspecific data show that for some plant species the SLA varies more than for others. We observed a tendency of the SLA to depend on latitude but in contrast to the interspecific results we did not observe an effect of altitude. The observed (in)-dependencies are the result of combining different growth-forms. For annual and perennial herbaceous plant species acclimated to different climates, the SLA depends on latitude while for woody plant species SLA depends on altitude. The effects of either latitude or altitude on SLA between plants species, having the same growth-form are similar. The negative dependencies of the SLA on latitude for species acclimated to different environments show that SLA is increased in cold climates, providing a compensatory mechanism, like observed at the interspecific level. However, the overall effect cannot be attributed specifically to an individual environmental variable. The observed overall independence might result from species-specific traits, in such a way that a particular environmental parameter is important for one species, and another parameter for a different species. In consequence the effect can not be observed for a variety of different plant species. In contrast to our results, earlier studies on phenotypic differences of plant species acclimated to different climates or temperatures either showed no results or a positive dependence of SLA on temperature (Atkin & Day 1990; Boese & Huner 1990; Xiong *et al.* 2000; Lee *et al.* 2005; Atkin *et al.* 2006; Galmes *et al.* 2007). Studies on light environment show that sun leaves have a lower SLA than shaded leaves (Bolstad *et al.* 1999; Turnbull *et al.* 2003).

The overall dependences of SLA at the intra- and interspecific level do not differ from each other. Thus for individuals of a plant species and between species similar changes in SLA in different climates compensate for the thermodynamic impact of

temperature on the metabolic rate. Linking the variations of SLA to differences in leaf size, our results show that at both the intra- and the interspecific level SLA is related to leaf size. These differences are expected to result from size-dependent variations in the construction of leaves (Niinemets 1999; Westoby *et al.* 2002), resulting in a high SLA of big leaves (Niklas *et al.* 2007). In addition, our results show that at the intraspecific level, plant species having big leaves have a high SLA, too. For annual herbaceous and woody plant species the dependence of SLA on leaf size is species-specific, reflecting that not all plants change SLA by changes in leaf size to the same extent (Milla *et al.* 2008). As shown before, the effect of leaf size on SLA does not differ between the intra- and interspecific level (Milla *et al.* 2008), so that for individuals of a plant species and between different species leaf construction follow similar rules. In addition we show for the first time that for plant species, acclimated to different climates, leaf size is related to latitude. This result applies for the growth-forms deciduous perennial herbaceous and woody plant species and is similar for species of the same growth-form. Perennial herbaceous and woody plant species both have bigger leaves in colder climates.

In conclusion, differences in SLA in different climates can compensate for the thermodynamic effect of temperature on the metabolic activity at the interspecific level. At the intraspecific level, the leaf size is related to latitude. In this case the effect of leaf size on SLA, and thus on metabolic rate, could also be considered a functional, compensatory adjustment to latitudinal effects.

## The intra- and interspecific impact of temperature on the specific leaf area and metabolic differences

Cordula Schmitz  
J. Theo M. Elzenga

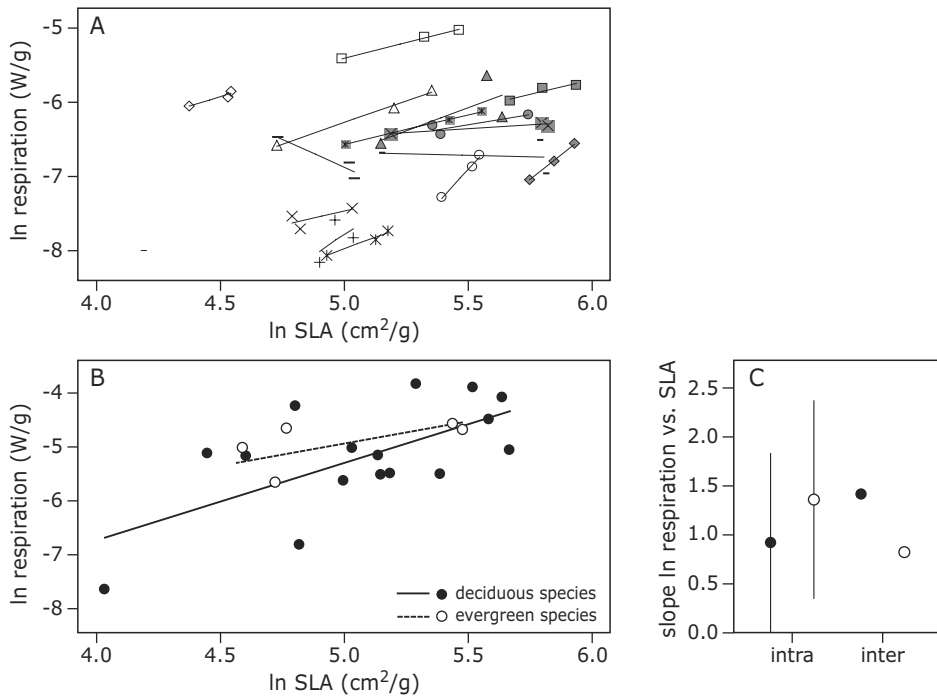
Differences in leaf surface to mass ratio of the leaves, the specific leaf area (SLA), have a major impact on the metabolic activity of plants. High surface area per unit leaf mass correlates with a high metabolic activity (Reich *et al.* 1997; Reich *et al.* 1999; Wright *et al.* 2004; Wright *et al.* 2005a; Wright *et al.* 2006). Acclimation and adaptation to a different temperature result in changes in the SLA of a single species and between different plant species, respectively (Wright *et al.* 2004; Wright *et al.* 2006; Campbell *et al.* 2007; Atkin *et al.* 2008). The short-term effect of temperature on the metabolic rate can be described by the thermodynamics of enzymatic reactions, given by the Arrhenius relation (Chapter 2; Gilooly *et al.* 2001; Brown *et al.* 2004). For plants exposed to a different temperature for a longer period, acclimation and adaptation can lead to adjustment of the metabolic rate, compensating the thermodynamic, short-term effect (Feder 1976; Somero 1978; Atkin & Tjoelker 2003; Clarke & Fraser 2004; O'Connor *et al.* 2007). We hypothesize that besides this physiological feedback regulation, temperature-dependent differences in the SLA provide an additional compensatory mechanism for thermodynamic effects of temperature on the basal metabolism (Chapter 5). Plants adapted to low temperatures could increase their basal metabolism by increasing the leaf surface area per unit leaf mass (dry weight).

While there is a wealth of information on interspecific differences, the effect of SLA on metabolic activity of plants acclimated to different temperatures (intraspecific) is hardly studied. The dependence of SLA on temperature is mainly examined by comparing different plants species from different climates. As several environmental factors such as solar radiation, precipitation and temperature vary gradually with latitude and affect the SLA, the combined effects are determined (Chapter 5). Here we show a metadata analysis on greenhouse studies, varying in temperature, from a total of publications (Appendix 2). We show the dependence of respiration on the specific leaf area (SLA) within a given plant species (acclimation) and taxonomic groups (adaptation) including deciduous and evergreen plant species. Furthermore, we examine the intra- and interspecific dependencies of SLA on acclimation and adaptation temperature. We hypothesize that if changes in SLA have compensatory effects on the basal metabolic rates, lower SLA values should be found at higher temperatures.

For the intraspecific data respiration is significantly, positively related to SLA (Figure Box 4.1A, Table Box 4.1). This dependence of respiration on SLA is similar for different included plant species (Table Box 4.1). Therefore variations in SLA at different temperatures provide a possibility for plants to adjust their metabolism to the prevailing conditions. The analysis of the interspecific data also show that respiration is dependent on SLA ( $N = 23$ ,  $R^2 = 0.36$ ,  $p < 0.005$ , regression coeff. = 1.10). In addition, the analysis shows that within the dataset on greenhouse cultured plants the SLA-dependent respiration, is independent of the growing conditions in the different studies ( $Df = 10$ ,  $F = 17.92$ ,  $p < 0.0001$ ). When the data are separated in the categories deciduous plants and evergreen plants, we observed a statistically significant similar dependence of respiration on SLA for both categories (Figure Box 4.1B, Table Box 4.1). The slope is 0.68 for deciduous and 1.42 for evergreens, indicating that respiration increases with increasing SLA. Variations in SLA resulting from acclimation (intraspecific) and adaptation (interspecific) may have different effects on respiration. However, the comparison of the intra- and interspecific relations show that the observed dependencies of  $\ln$  respiration on  $\ln$  SLA are similar within a single species and between different species, as the mean is within the 95% confidence interval (Figure Box 4.1C). These results imply that genetically-based differences in SLA between species, have similar effects on the metabolism as differences at the intraspecific level. Therefore, temperature-dependent morphological variations reflected by the SLA, such as differences in the amount of parenchyma cells, cell sizes, stomata density as well as nitrogen or chlorophyll content, result in differences in respiration (Kriedemann 1986; Boese & Huner 1990; Beerling & Chaloner 1993; Reich *et al.* 1997; Poorter & Evans 1998; Kundu & Tigerstedt 1999; Reich *et al.* 1999; Atkin *et al.* 2006a).

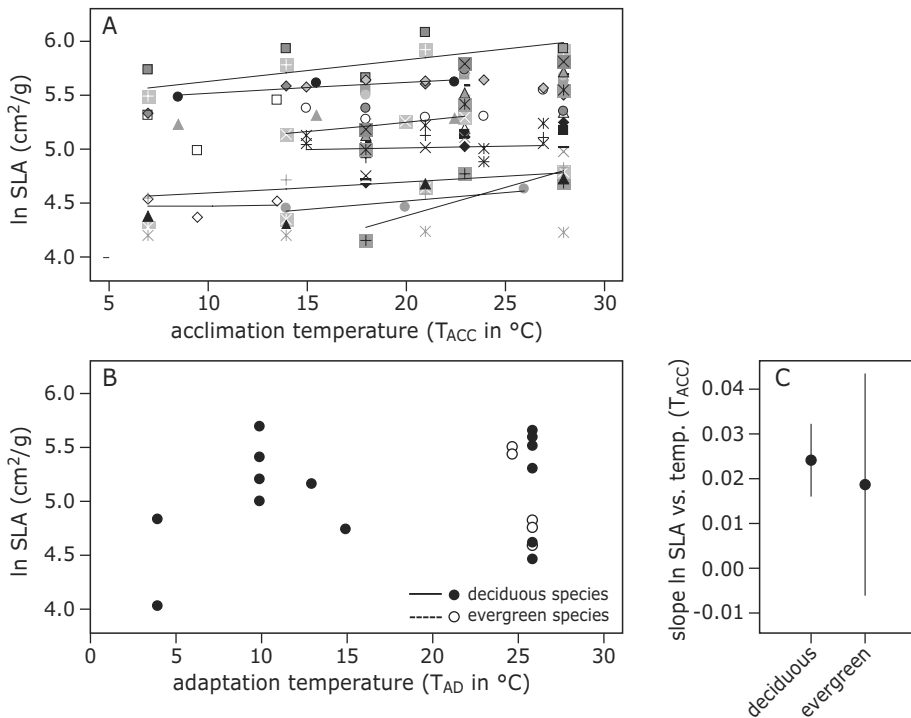
		Variable		Analysis					
Dependent		Independent		Multivariate ANCOVA			Homogeneity of slope		
		Categorical	Continuous	Df	F	p	Df	F	p
Intra	$\ln R$ (Watt/g)	species	$\ln SLA$ (cm <sup>2</sup> /g)	15	2.83	<0.03	14	1.88	0.119
Inter	$\ln R$ (Watt/g)	dec/ever	$\ln SLA$ (cm <sup>2</sup> /g)	2	7.37	<0.005	1	0.81	0.381
Intra	$\ln SLA$ (cm <sup>2</sup> /g)	species	temperature	35	3.11	<0.0001	34	1.57	0.066
Inter	$\ln SLA$ (cm <sup>2</sup> /g)	dec/ever	temperature	2	3.16	0.068			

**Table Box 4.1** Statistics on the intra- and interspecific dependencies of  $\ln$  respiration on  $\ln$  SLA. The intraspecific relations reflect the within a plant species and the interspecific relations the between species separated to deciduous (dec) and evergreen (ever) plants dependencies. The multivariate ANCOVA indicate the significance ( $p < 0.05$ ) of the relations for the included categories and the homogeneity of slope significant differences ( $p < 0.05$ ) between the observed relations.



**Figure Box 4.1** (A) Intra- and (B) interspecific  $\ln$  -  $\ln$  dependence of mass-normalized respiration (given by the respiration measurement at the acclimation temperature for intraspecific data and measurement at adaptation temperature for interspecific data) to specific leaf area (SLA in  $\text{cm}^2/\text{g}$ ). For intraspecific data each symbol represents a species, listed in the legend below, so that the regression shows the species-specific response. For interspecific data symbols are separated to deciduous and evergreen plants. Figure (C) shows the intra- and interspecific mean scaling exponents of the  $\ln$  -  $\ln$  dependencies for deciduous and evergreen plants, the error bars show the 95% confidence interval.

- |                                  |                                 |
|----------------------------------|---------------------------------|
| ■ <i>Acacia aneura</i>           | △ <i>Acacia melanoxylon</i>     |
| - <i>Achillea millefolium</i>    | - <i>Achillea ptarmica</i>      |
| □ <i>Arabidopsis thaliana</i>    | ○ <i>Betula papyrifera</i>      |
| ◇ <i>Bromus erectus</i>          | ■ <i>Bromus ramosus</i>         |
| ⊠ <i>Chlorophytum</i> sp.        | ⊠ <i>Cistus ladanifer</i>       |
| ▲ <i>Cistus laurifolius</i>      | □ <i>Colobanthus quitensis</i>  |
| ● <i>Dactylus golmerata</i>      | ◇ <i>Deschampsia antarctica</i> |
| ■ <i>Eucalyptus delegatensis</i> | ◆ <i>Eucalyptus dumosa</i>      |
| ▲ <i>Festuca pratensis</i>       | ● <i>Fragaria</i> sp.           |
| ■ <i>Geum rivale</i>             | ● <i>Geum urbanum</i>           |
| × <i>Larix laricina</i>          | × <i>Luzula acutifolia</i>      |
| ✱ <i>Picea mariana</i>           | + <i>Pinus banksiana</i>        |
| - <i>Plantago euryphylla</i>     | - <i>Plantago lanceolata</i>    |
| ▲ <i>Plantago major</i>          | ● <i>Poa costiniana</i>         |
| ■ <i>Poa trivialis</i>           | ◇ <i>Populus tremuloides</i>    |
| ✱ <i>Quercus ilex</i>            | + <i>Quercus suber</i>          |
| ⊠ <i>Silene dioica</i>           | ⊠ <i>Silene uniflora</i>        |



**Figure Box 4.2** (A) Intra - and (B) interspecific dependencies of  $\ln$  SLA on acclimation temperature ( $T_{\text{ACC}}$ ), given by the prevailing temperature for intraspecific data and on adaptation temperature ( $T_{\text{ADA}}$ ) given by the mean growing season temperature in the region of origin for interspecific data. For intraspecific data each symbol represents a species, the same given in Figure Box 4.1A (see legend). Interspecific data are separated to deciduous and evergreen plants. The average slope of the relations of SLA to acclimation temperature is shown in Figure (C).

That metabolic processes are dependent on the surface to biomass ratio of leaves at the interspecific level has been shown in several studies (Reich *et al.* 1999; Wright *et al.* 2004; Wright *et al.* 2005a; Poorter *et al.* 2009). These studies show that increasing the SLA results in increased respiration.

Here we showed for the first time, that the respiration of plants acclimated to different temperatures is positively related to SLA, a relation that is similar to the interspecific dependence. Similarities of the biomass scaling relation within and between species are also shown for animals (Kleiber 1947; West *et al.* 1997; Terblanche *et al.* 2004; Terblanche *et al.* 2005; Glazier 2005; Glazier 2006; Terblanche *et al.* 2007)

As variations in SLA have an impact on metabolic processes, temperature-dependent differences in SLA could provide a compensatory mechanism for thermodynamically induced variations in the metabolic rate. The intraspecific dependence of

SLA on temperature show that variations in temperature are accompanied by differences in SLA (Table Box 4.1). The observed relations of SLA to temperature are similar for the different species (Table Box 4.1). The slopes range from -0.003 to 0.063, the average 0.023, showing that SLA, on average, increases with increasing temperature. Thus overall thermal acclimation leads to a high surface to biomass ratio at higher temperatures, and in consequence to an increased metabolic rate.

The interspecific relation of the SLA to average growing season temperature shows that SLA is independent of mean growing season temperature. The independence of SLA on temperature for greenhouse data does not result from combining data of different studies varying in growing conditions, since we observe the same results considering the effect of temperature on the SLA within publications ( $Df = 3$ ,  $F = 1.60$ ,  $p = 0.20$ ). Further, we separated the data into deciduous and evergreen plants, to exclude the effect of combining these two taxonomic groups, but still observed a temperature-independence of the SLA (Table Box 4.1). This independence might be the result of the scarcity of the data available, because plants have to be grown at a temperature close to the temperature in the region of origin. In addition the greenhouse data on evergreen plants only cover high adaptation temperatures ranging from 24.8 to 26°C, so that the results are not representative for the whole range of different temperatures. Moreover, the  $R^2$  of 0.03 for deciduous reflect the high variance in SLA between species, so that the effect of temperature might be obscured by the included species.

Earlier studies show that the SLA differs between species adapted to different temperatures (Reich *et al.* 1997; Reich *et al.* 1999; Wright *et al.* 2005a; Wright *et al.* 2006; Poorter *et al.* 2009). However, those studies are based on field data, so that differences in the SLA reflect the impact of climates, not just of temperature. Field data are influenced by other environmental factors such as solar radiation, wind-speed or water and nutrient availability, which have been shown to potentially affect the SLA (Woodward 1983; Poorter & Evans 1998; Bolstad *et al.* 1999; Wright *et al.* 2006). Further, relations of SLA to different climates available in the literature differ considerably. In warm climates SLA is low for deciduous plants, but high for evergreens (Chapter 5; Wright *et al.* 2004; Wright *et al.* 2005a). In contrast, other studies showed that plants adapted to warm climates have a high SLA (Poorter *et al.* 2009). The variations in SLA between plant species and the observed differences in the relation for deciduous and evergreen plants, reflect the dependence of SLA on plant traits (Kitajima 1994; Reich *et al.* 1999). Studies detecting low SLA in warm climates are on data covering a broad temperature range including extreme temperatures below -15°C (Wright *et al.* 2005a). As extreme temperatures cover a low range of plant taxa and SLA is taxa-dependent, including extreme temperatures in the data set might result in a different regression coefficients.

Beside differences in respiration, differences in SLA are also accompanied by several costs and benefits like e.g. leaf life-span, structure or nitrogen use efficiency (Reich *et al.* 1997; Wright *et al.* 2004; Wright *et al.* 2005a; Wright *et al.* 2005b).

Modification of the SLA to compensate for temperature effects on the metabolic rate is probably highly constrained by other, more important, species-specific traits. In conclusion, contrary to our hypothesis, at the interspecific level SLA is independent of temperature and at the intraspecific level high temperature results in high SLA, instead of low SLA, which would compensate the metabolic rate for the short-term effect of temperature on enzymatic reactions.



